AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

SUSCEPTIBILITY

Manogepix (APX001A) Displays Potent *In Vitro* Activity against Human Pathogenic Yeast, but with an Unexpected Correlation to Fluconazole MICs

Maiken Cavling Arendrup,[a,b,c] Karin Meinike Jørgensen[a]

[a]Unit of Mycology, Statens Serum Institut, Copenhagen, Denmark
[b]Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark
[c]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

ABSTRACT Manogepix (APX001A) is the active moiety of the novel drug candidate fosmanogepix (APX001). We previously reported the broad-spectrum activity of manogepix but also observed a correlation between increased manogepix and fluconazole MICs. Here, we extended this study and included isolates with acquired fluconazole resistance. Isolates ($n = 835$) were identified using CHROMagar, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), and, when needed, internal transcribed spacer (ITS) sequencing. EUCAST E.Def 7.3.1 susceptibility testing included manogepix, amphotericin B, anidulafungin, micafungin, fluconazole, and voriconazole. Manogepix wild-type-upper-limit (WT-UL) values were established following EUCAST principles for the epidemiological cutoff value (ECOFF) setting allowing wild-type/non-wild-type classification. Drug-specific MIC correlations were investigated using Pearson's correlation. Manogepix modal MICs were low (range, 0.004 to 0.06 mg/liter against 16/20 included species). Exceptions were *Candida krusei* and *Candida inconspicua* and, to a lesser extent, *Candida kefyr* and *Pichia kluyveri*. The activity was independent of Fks echinocandin hot spot alterations ($n = 17$). Adopting the WT-UL established for *Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis*, 14/724 (1.9%) isolates were non-wild type for manogepix. Twelve of these (85.7%) were also non-wild type for fluconazole. A statistically significant correlation was observed between manogepix and fluconazole MICs for *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* (Pearson's $r = 0.401$ to 0.575) but not between manogepix and micafungin or amphotericin B MICs for any species except *C. tropicalis* ($r = 0.519$ for manogepix versus micafungin). Broad-spectrum activity was confirmed for manogepix against contemporary yeast. However, a 1 to 4 2-fold dilutions increase in manogepix MICs is observed in a subset of isolates with acquired fluconazole resistance. Further studies on the potential underlying mechanism and implication for optimal dosing are warranted.

KEYWORDS APX001A, EUCAST, MGX, antifungal susceptibility testing, fluconazole, manogepix

Manogepix (MGX, formerly APX001A and E1210) is the active moiety of the first-in-class small-molecule drug candidate fosmanogepix (APX001). In multiple nonclinical studies, manogepix has shown broad-spectrum activity against *Candida* (except *Candida krusei* but including the multidrug-resistant emerging species *Candida auris*), *Aspergillus*, and other difficult-to-treat molds (1–6). Fosmanogepix is currently in multiple phase 2 clinical trials for candidemia, as well as infections caused by *C. auris*, *Aspergillus*, and rare molds.

Citation Arendrup MC, Jørgensen KM. 2020. Manogepix (APX001A) displays potent *in vitro* activity against human pathogenic yeast, but with an unexpected correlation to fluconazole MICs. Antimicrob Agents Chemother 64:e00429-20. https://doi.org/10.1128/AAC.00429-20.

Address correspondence to Maiken Cavling Arendrup, maca@ssi.dk.

Received 5 March 2020
Returned for modification 15 April 2020
Accepted 25 April 2020
Accepted manuscript posted online 4 May 2020
Published 23 June 2020

We included manogepix in our prospective EUCAST antifungal susceptibility testing of bloodstream isolates referred as part of the nationwide fungemia surveillance program in Denmark in 2016 to 2017 to generate population-based contemporary EUCAST MIC data for manogepix (7). We found that manogepix was highly active against yeast bloodstream isolates. However, while no evidence of cross-resistance was observed with echinocandins or amphotericin B, there was a correlation noted between manogepix and fluconazole MICs across several yeast species. This was unexpected because the two compounds target two different targets and pathways. Manogepix inhibits the conserved fungal inositol acyltransferase enzyme encoded by *GWT1*, thereby preventing trafficking and anchoring mannoprotein to the outer cell wall. Fluconazole and other azoles target the lanosterol 14-alpha demethylase enzyme encoded by *ERG11*, thereby interfering with the ergosterol synthesis, an essential compound of the fungal cell membrane. Ultimately, both consequently compromise fungal growth. Resistance to azoles is often multifactorial and involves one or several *erg11* target gene mutations (affecting drug affinity for the enzyme), target gene upregulation (thereby outcompeting the number of drug molecules), or expression of drug efflux pumps (lowering the intracellular amount of drug) (8, 9). Strains with 1 to 5 2-fold dilutions steps elevated MIC values to manogepix have been identified *in vitro* in *Candida albicans*, *Candida glabrata*, and *Candida parapsilosis*, but not in *C. auris* or *Candida tropicalis* (10). The spontaneous mutation rate was low, 3×10^8 to $\leq 1.85 \times 10^8$. In some of the mutant strains, a valine-to-alanine alteration, V162A in *C. albicans* and V163A in *C. glabrata*, respectively, was found in the Gwt1 target protein that resulted in increased manogepix MIC values when expressed in *C. albicans* and *Saccharomyces cerevisiae*. Other mutants were isolated with increased MGX MIC values that did not show mutations in *GWT1* (10).

In this study, we investigated the *in vitro* susceptibility to manogepix, fluconazole, voriconazole, and other comparators against yeast isolates from blood and other sources received at the reference laboratory in Denmark in 2018. The objectives were 2-fold. The first was to continue and extend the surveillance of the *in vitro* activity of manogepix against contemporary yeast isolates. The second was to study more isolates with non-wild-type susceptibility to licensed antifungal agents. The latter was done by including isolates from sources other than blood, in which acquired resistance, in general, is more common than among unique (and often initial) bloodstream isolates referred for epidemiological purposes (11).

RESULTS

Manogepix against quality control strains. *C. albicans* CNM-CL-F8555, *C. parapsilosis* ATCC 22019, and *C. krusei* ATCC 6258 were tested 19, 62, and 65 times, respectively, during the study period using EUCAST methodology (12). The MIC results against *C. albicans* CNM-CL-F8555 were as follows (modal MIC and MIC_{50} [range]): 0.03 mg/liter and 0.03 mg/liter (0.008 to 0.06 mg/liter) with 18/19 (94.7%) MICs within the range of 0.016 to 0.06 mg/liter. The MICs against *C. parapsilosis* ATCC 22019 were 0.03 mg/liter and 0.03 mg/liter (0.016 to 0.125 mg/liter), with 60/62 (96.8%) MICs within the range of 0.016 to 0.03 mg/liter. Finally, the MICs against *C. krusei* ATCC 6258 were 0.5 mg/liter on two occasions and >0.5 mg/liter (outside the tested concentration range) for the rest ($n = 63$).

Manogepix activity against contemporary *Candida*, *Saccharomyces*, and *Pichia* isolates. On a milligram per liter basis, manogepix was highly active against 16 of 20 species (modal MICs between 0.004 and 0.06 mg/liter), with *Candida dubliniensis* being the most susceptible organism and *C. glabrata* the least (Table 1). Manogepix MICs were higher against four species, including *C. krusei* and the related species *Candida inconspicua* (MICs >0.5 mg/liter for all but one *C. krusei* isolate) and, to a lesser extent, against *Candida kefyr* (MIC range, 0.125 to >0.5 mg/liter) and *Pichia kluyveri* (MICs of 0.125 and 0.5 mg/liter, respectively). These four species included 8.4% (70/835) of the isolates. Wild-type-upper-limit (WT-UL) values were set statistically (including 97.5%

TABLE 1 *In vitro* activity of manogepix against 835 yeast isolates by species and for isolates harboring echinocandin target gene mutations[a]

	Characteristics of manogepix *in vitro* activity													
	MIC (mg/liter)										WT-UL ECOFFinder[b]			
Species (no.)	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	>0.5	97.5%	99.0%	WT-UL visual	Non-wild type (*n*)
Low-MIC species (765)														
C. albicans (402)		34	237	111	15	3	1	1			0.016	0.016	0.03	5
fks mutant *C. albicans*		1	2	2			1	1						
C. dubliniensis (48)	1	27	19				1				0.008	0.008	0.016	1
fks mutant *C. dubliniensis*			1											
C. glabrata (188)	1		2	6	40	114	22	**2**			0.125	0.125	0.125	2
fks mutant *C. glabrata*						5	1							
S. cerevisiae (9)					4	4	1				ND[c]	ND	ND	
Saccharomyces telluris (1)							1							
C. parapsilosis complex (45)			4	17	20	2	1	**1**			0.06	0.06	0.06	2
C. parapsilosis (39)			2	16	18	2	1				0.06	0.06	0.06	1
Candida metapsilosis (2)			2											
C. orthopsilosis (4)				1	2			**1**						1
C. tropicalis (41)		3	31	5	**2**						0.016	0.016	0.016	2
fks mutant *C. tropicalis*					1									
C. lusitaniae (12)		1	3	8							ND	ND	ND	
C. guilliermondii (9)	2	3	2	1		1					ND	ND	ND	(1)
Candida fermentati (2)	1			1										
Candida pelliculosa (1)	1													
Candida utilis (2)		1			1									
Candida species[d] (4)	1		3											
Pichia salicaria (1)				1										
High-MIC species (70)														
C. krusei (54)								1		53	NP	NP	ND	
fks mutant *C. krusei*										2				
C. inconspicua (2)										2				
C. kefyr (12)							4	3	4	1	ND	ND	ND	(1)
P. kluyveri (2)							1		1					
Total (835)	7	69	301	150	82	124	33	8	5	56				

[a]The modal MIC is underlined for species represented by more than 10 isolates. MICs for *fks* mutant isolates are indicated in gray font. Non-wild-type isolates are indicated in bold font.
[b]WT-UL, wild-type upper limit determined visually and statistically using the ECOFFinder program, which includes 97.5% and 99% of the isolates, respectively.
[c]ND, not done. NP, not provided; the ECOFFinder program requires at least one isolate with MIC at least one step above the modal MIC to calculate an upper limit.
[d]Five species could not be identified by sequencing; three were a new species closely related to *Candida blankii*, and two were potentially *C. metapsilosis* but with an unreliable identification.

and 99% of the isolates) and visually for species represented by at least 15 isolates and agreed within 1 2-fold dilution.

The following 17 *Candida* isolates harbored echinocandin *fks* target gene-encoded hot spot alterations: Fks1 P1354P/S ($n = 2$), Fks1 P1354S ($n = 4$), and Fks1 R1361R/S ($n = 1$) for *C. albicans* ($n = 7$); Fks1 S645P ($n = 1$) for *C. dubliniensis* ($n = 1$); Fks1 F625S ($n = 1$), Fks2 F659S ($n = 1$), Fks2 F659del ($n = 1$), Fks2 S663F ($n = 1$), Fks2 S663F ($n = 1$), and Fks2 S663P ($n = 1$) for *C. glabrata* ($n = 6$); Fks1 S659F ($n = 1$) and Fks1 S659S/F ($n = 1$) for *C. krusei* ($n = 2$); and Fks1 F650S ($n = 1$) for *C. tropicalis* ($n = 1$). The manogepix *in vitro* activity against these mutant isolates was similar to their wild-type counterparts with the exception of two *C. albicans* with manogepix MICs of 0.125 and 0.25 mg/liter, respectively, which were also pan-azole resistant (Table 1).

Comparison of *in vitro* activity of manogepix to other antifungal agents. Manogepix was more active than fluconazole with respect to MIC values or the number of non-wild-type isolates, with the exception of *C. kefyr*, where MGX was equally active (Table 2). Manogepix was also more active than voriconazole and amphotericin B except against *C. krusei* and *C. kefyr* and in displaying comparable activity to that of voriconazole against *C. parapsilosis* and *Candida lusitaniae*. Finally, manogepix was more active against *C. parapsilosis* complex isolates and *Candida guilliermondii*, whereas anidulafungin was more active against *C. krusei* and *C. kefyr* (Table 2).

TABLE 2 Summary of the *in vitro* activity of manogepix and comparators against contemporary yeast isolates

	In vitro activity measures for:																							
	Manogepix				Fluconazole				Voriconazole				Amphotericin B				Anidulafungin				Micafungin			
Species	MIC_{50}	MIC_{90}	Range	Non-WT[a]	MIC_{50}	MIC_{90}	Range	Non-WT	MIC_{50}	MIC_{90}	Range	Non-WT	MIC_{50}	MIC_{90}	Range	Non-WT	MIC_{50}	MIC_{90}	Range	Non-WT	MIC_{50}	MIC_{90}	Range	Non-WT
C. albicans	0.008	0.016	0.004 to 0.25	5	0.125	0.25	<0.06 to >64	23	<0.004	0.008	<0.004 to >4	21	0.25	0.25	0.06 to 0.5	0	0.004	0.008	<0.004 to 0.06	3	0.016	0.016	<0.004 to 0.125	6
C. dubliniensis	0.004	0.008	0.002 to 0.125	1	0.125	0.5	0.06 to 32	2	0.008	0.016	<0.004 to 0.25	2	0.06	0.06	0.03 to 0.125	0	0.008	0.016	0.008 to 0.25	1	0.03	0.03	0.008 to 2	1
C. glabrata	0.06	0.125	0.002 to 0.25	2	4	32	0.5 to >64	21	0.06	0.5	0.0084	15	0.25	0.5	0.03 to 0.5	0	0.03	0.03	0.008 to 1	6	0.016	0.03	0.008 to 0.5	6
S. cerevisiae	0.06	0.125	0.03 to 0.125	(0)	8	32	2 to 32	ND	0.125	0.5	0.06 to 0.5	(0)	0.125	0.25	0.03 to 0.25	0	0.06	0.125	0.03 to 0.125	(0)	0.25	0.25	0.06 to 0.25	0
C. krusei	>0.5	>0.5	0.25 to >0.5	ND	32	43	8 > 64	(0)	0.25	0.5	0.125 to >4	1	0.5	1	0.5 to 1	0	0.03	0.06	0.016 to 0.25	2	0.125	0.25	0.06 to 4	1
C. parapsilosis	0.03	0.03	0.008 to 0.125	1	0.5	2	0.25 to 64	1	0.016	0.03	<0.004 to 0.5	1	0.5	0.5	0.125 to 0.5	0	1	2	0.25 to 2		1	2	0.25 to 4	0
C. tropicalis	0.008	0.016	0.004 to 0.03	2	0.5	1	0.125 to 8	1	0.03	0.03	0.008 to 0.5	1	0.25	0.5	0.125 to 0.5	0	0.016	0.03	0.004 to 0.25	1	0.03	0.03	0.016 to 1	1
C. kefyr	0.25	0.5	0.125 to >0.5	ND	0.25	0.5	0.25 to 8	1	0.008	0.016	0.008 to 0.06	(1)	0.5	0.5	0.25 to 1	0	0.016	0.03	0.016 to 0.06	(0)	0.06	0.06	0.03 to 0.125	0
C. lusitaniae	0.016	0.016	0.004 to 0.016	0	0.5	0.5	0.25 to 0.5	0	0.008	0.016	<0.004 to 0.016	(0)	0.125	0.25	0.06 to 1	1	0.03	0.06	0.03 to 0.125	(0)	0.06	0.125	0.03 to 0.25	0
C. guilliermondii	0.004	0.06	0.002 to 0.06	(1)	8	>64	2 to >64	ND	0.125	4	0.03 to 4	ND	0.25	1	0.125 to 1	1	0.5	1	0.25 to 1	(0)	0.25	0.5	0.125 to 0.5	0

[a]Non-WT, number of non-wild-type isolates.

Adopting the highest manogepix WT-UL of the three WT-ULs determined (in all cases, the visual WT-UL), 14 isolates were classified as non-wild type for manogepix (Table 1). Twelve of these (85.7%) were also non-wild type for fluconazole, including 4/5 *C. albicans* isolates (fluconazole MICs of 2, 8, >32, and 64 mg/liter), 1/1 *C. dubliniensis* isolate (fluconazole MIC of 32 mg/liter), 2/2 *C. glabrata* isolates (fluconazole MICs of 64 and >64 mg/liter, respectively), 2/2 *C. parapsilosis* complex isolates (*C. parapsilosis* and *Candida orthopsilosis*, respectively, both with fluconazole MICs of 64 mg/liter) and 1/2 non-wild-type *C. tropicalis* isolates (fluconazole MIC of 8 mg/liter) (Table 3). Additionally, high fluconazole MICs were observed for the *C. guilliermondii* (fluconazole MIC of 64 mg/liter) and *C. kefyr* (fluconazole MIC of 8 mg/liter) isolates with the highest manogepix MICs (0.06 mg/liter and >0.5 mg/liter, respectively) (Table 1). Whereas elevated fluconazole MICs were observed for the vast majority of isolates with elevated/high manogepix MICs, the opposite was not the case. Of note, three *C. albicans* isolates deemed fluconazole resistant due to a heavy trailing growth (with 50% to 75% growth compared to the growth control at all fluconazole concentrations tested) remained highly manogepix susceptible (Table 3).

Finally, Pearson's correlation was used to compare the MICs at the isolate level (Table 4). A moderate correlation was observed between manogepix and fluconazole MICs for all species represented by more than 10 isolates (except *C. krusei*), which was statistically significant for *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. Similarly, a significant correlation was found between manogepix and voriconazole, although it was only weak against *C. albicans*, for which the voriconazole MIC distribution was truncated, with more than half of the isolates having a voriconazole MIC of <0.004 mg/liter (MIC_{50} <0.004 mg/liter; Table 2). In contrast, only a weak or very weak correlation was observed when manogepix was compared to micafungin and amphotericin B, with the exception of manogepix and micafungin against *C. tropicalis*.

DISCUSSION

This study confirms the broad-spectrum activity of manogepix against most human pathogenic yeast species. Overall, the wild-type populations of 16/20 species were intrinsically highly susceptible, with modal MICs ranging from 0.004 to 0.06 mg/liter. These species included the majority (91.6%) of isolates sent to our reference laboratory, and the susceptibility was independent of the presence or absence of *fks*-mediated echinocandin resistance. Our study also confirms that some species are intrinsically nonsusceptible to manogepix, including *C. krusei* and the closely related species *C. inconspicua*. These two species constituted 7.1% of our isolates.

The interlaboratory reproducibility of EUCAST MIC determination for manogepix appears to be high, as all species' specific modal MICs determined in our previous study were confirmed (7). It also suggests that for most species, EUCAST and CLSI generate similar MICs. Thus, modal MICs by the CLSI method for *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. tropicalis*, and *C. lusitaniae* were either identical to or 1 2-fold dilution apart from the EUCAST modal MICs presented here in a recent study with 1,340 *Candida* species isolates (6, 13).

We previously reported an unexpected correlation between intrinsic susceptibility to manogepix and fluconazole (7). This was documented by a linear relationship between modal MICs for fluconazole and manogepix for the six most prevalent *Candida* species (*C. dubliniensis*, *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei*) and *C. lusitaniae*. The exceptions were the species *C. guilliermondii* and *C. auris* for which manogepix modal MICs were low (0.008 to 0.016 mg/liter) but fluconazole modal MICs were high (4 mg/liter and 512 mg/liter, respectively). But interestingly, it was also found that 4/4 isolates with manogepix MICs above the WT-UL were also non-wild type to fluconazole, suggesting that there is a single resistance mechanism responsible for the elevation of both manogepix and fluconazole MICs. In this study, we further substantiated this observation. First, 85.7% of the isolates found non-wild type to manogepix were also non-wild type for fluconazole, and for the remaining two isolates, the manogepix MIC was only 1 2-fold dilution above the WT-UL, suggesting either presence

TABLE 3 Checkerboard presentation of manogepix and fluconazole MICs for *Candida* species represented by ≥10 isolates[a,b]

Spp. and manogepix MIC (mg/L)	Fluconazole MIC (mg/L)													
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥32	64	≥64	>64
C. albicans														
0.004	1	18	12	1	1									1*
0.008	9	134	86	6										2*
0.016	3	54	46	2		1	2		1	1	1			
0.03	1		3	2			1	4				2		2
0.06				1		**1**		**1**						
0.125													**1**	
0.25												**1**		
C. dubliniensis														
0.002		1												
0.004	3	16	6	1					1					
0.008		9	7	3										
0.125										**1**				
C. glabrata														
0.002							1							
0.008						1	1							
0.016				1	1	2	2							
0.03						20	20							
0.06						39	55	9	4	2		4		1
0.125						1	6	4		2		8	1	1
0.25												**1**		**1**
C. krusei														
0.25								1						
>0.5									18	21		12		2
C. parapsilosis*														
0.008				2										
0.016				11	4	1								
0.03			1	7	6	4								
0.06				2										
0.125												**1**		
C. tropicalis														
0.004			3											
0.008		3	8	13	6	1								
0.016			1	3	1									
0.03				1				**1**						
C. kefyr														
0.125			2	2										
0.25			3											
0.5			2	2										
>0.5								**1**						
C. lusitaniae														
0.004			1											
0.008			2	1										
0.016			2	6										

[a]The horizontal lines indicate the WT-UL values determined for manogepix in this study. The solid vertical lines indicate the EUCAST ECOFFs, and the stippled vertical lines are tentative EUCAST ECOFFs for fluconazole (valid from 2 April 2020). Isolates non-wild type to both manogepix and fluconazole are highlighted in bold.

[b]Symbols: *, heavy trailing phenotype with <50% growth inhibition over a wide concentration range (0.125 to 64 mg/liter); **, *C. parapsilosis sensu stricto* only.

of a low-grade resistance mechanism or that the MIC elevation in these isolates is explained by technical variation rather than acquired resistance. Second, a statistically significant correlation was observed between manogepix and fluconazole MICs for isolates within each individual *Candida* species, except the intrinsically resistant *C. krusei* and those represented by few isolates (*C. kefyr* and *C. lusitaniae*).

TABLE 4 Detailed summary of species-specific pairwise MIC comparisons[a,b]

Species (no.)	Data for comparison of: MGX vs FCZ			MGX vs VCZ			FCZ vs VCZ			MGX vs MFG			MGX vs AMB		
	P	*r*	Corr. class.	*P*	*r*	Corr. class.	*P*	*r*	Corr. class.	*P*	*r*	Corr. class.	*P* value	*r*	Corr. class.
C. albicans (402)	<0.0001	0.401	M	<0.0001	0.267	W	<0.0001	0.566	M[c]	<0.0001	0.203	W	<0.0001	0.206	W
C. albicans excl. FLC-resistant isolates with trailing phenotype (399)	<0.0001	0.488	M	<0.0001	0.329	W	<0.0001	0.466	M[c]	<0.0001	0.239	W	<0.0001	0.224	W
C. dubliniensis (48)	<0.0001	0.575	M	<0.0001	0.552	M	<0.0001	0.799	S	0.135	0.219	W	0.071	0.263	W
C. glabrata (188)	<0.0001	0.479	M	<0.0001	0.473	M	<0.0001	0.931	VS	0.019	0.173	VW	0.002	0.230	W
C. krusei (54)	ND	ND		ND	ND		<0.0001	0.773	S	ND	ND		0.503	0.093	VW
C. parapsilosis (39)	0.001	0.504	M	0.0001	0.572	M	<0.0001	0.886	VS	0.167	−0.226	W	0.765	0.050	VW
C. tropicalis (41)	0.006	0.420	M	0.065	0.291	W	<0.0001	0.847	VS	0.001	0.519	M	0.029	0.342	W
C. kefyr (12)	0.078	0.528	M	0.046	0.584	M	0.0002	0.878	VS	0.312	0.319	W	0.255	0.357	W
C. lusitaniae (12)	0.093	0.506	M	0.484	0.224	W	0.603	−0.167	VW	0.617	−0.161	VW	0.450	0.241	W

[a]MGX, manogepix; FCZ, fluconazole; VCZ, voriconazole; MFG, micafungin; AMB, amphotericin B; corr. class., correlation classification; VW, very weak; W, weak; M, moderate; S, strong; VS, very strong; ND, not done. MGX MIC distribution truncated at 0.5 mg/liter.

[b]*P* and *r* determined using Pearson's correlation on $\log_2$-transformed MICs. *P* values regarded as significant (<0.05) and associated *r* values are indicated in gray shading.

[c]The MIC distribution for voriconazole against *C. albicans* was truncated with the voriconazole MIC <0.004 mg/liter for more than half of the isolates. This may have lowered the Pearson's correlation coefficient.

The mechanism behind the concomitant increase in MIC values for manogepix and fluconazole is unexplained. Manogepix targets Gwt1 and not Erg11, which is targeted by the azoles. Manogepix susceptibility was uniform against Indian *C. auris* isolates, although that vast majority were highly fluconazole resistant (7). Fluconazole resistance in Indian *C. auris* has been linked to two specific Erg11 amino acid substitutions, Y132 and K143, but significant overexpression of *erg11* has not been found, unlike what is the case in fluconazole-resistant *C. albicans* and some other species (8, 14). This might suggest that drug export via a common efflux pump induced by fluconazole resistance is more likely the underlying mechanism of the observed drug cross-resistance in our isolates. This hypothesis is supported by recent findings in a study where increased MICs to manogepix were identified *in vitro* in *C. albicans*, *C. glabrata*, and *C. parapsilosis* (10). In *C. albicans* and *C. glabrata* but not *C. parapsilosis*, a mutation was identified that resulted in a valine-to-alanine alteration (V162A in *C. albicans* and V163A in *C. glabrata*, respectively) in the Gwt1 target protein. This alteration resulted in 4 to 5 2-fold dilutions increased manogepix MIC values versus the isogenic starting strains. In addition, when the V163A mutation was inserted into wild-type *C. glabrata*, the MIC value of the transformant was also elevated 5 2-fold dilutions, demonstrating that this change was necessary and sufficient for the altered MIC values (10). Of note, cross-resistance to fluconazole was examined in five mutant strains with and without *gwt1* mutations. Fluconazole susceptibility remained unchanged in four of these, whereas 2 2-fold dilutions MIC elevation was observed in one *C. parapsilosis* mutant with a wild-type *gwt1* target gene (10). Thus, it appears that elevated manogepix values in mutants isolated *in vitro* are rarely associated with an elevation of fluconazole MICs or resistance, whereas clinical isolates with elevated manogepix MICs despite no prior manogepix exposure are universally cross resistant to fluconazole.

The clinical implication of the potential for cross-resistance remains to be understood. In our study, the manogepix elevation was limited to 1–3 2-fold dilutions above the WT-UL. If the future standard dose is sufficient to encompass this MIC elevation or if the tolerability profile allows an appropriate dose escalation option, such cross-resistance may be successfully treated with manogepix.

In conclusion, we confirm previous findings that manogepix displays a promising broad-spectrum activity against most *Candida*, *Saccharomyces*, and *Pichia* species with the exception of *C. krusei*. No cross-resistance was observed with echinocandin or amphotericin B. However, a 1 to 3 2-fold dilutions elevation of MGX MICs is observed in some isolates with acquired fluconazole resistance. Further studies on the potential underlying mechanism and implication for optimal dosing are warranted. Finally, this

study also suggests that EUCAST susceptibility testing of MGX is robust and in close agreement with the CLSI method. This suggests that the establishment of mutual breakpoints may be feasible.

MATERIALS AND METHODS

A total of 835 isolates obtained in 2018 and derived from blood ($n = 491$), unspecified drains ($n = 87$), abdomen/gallbladder ($n = 60$), oral cavity/esophagus ($n = 32$), pigtail catheter/urine ($n = 26$), pleura ($n = 19$), bone ($n = 18$), or other sources each represented by ≥11 samples ($n = 102$) were included. The blood isolates were obtained as part of the nationwide Danish surveillance program and thus represent a contemporary national and population-based yeast isolate collection.

Susceptibility testing. EUCAST MICs were determined following E.Def 7.3.1 methodology (12). Manogepix (APX001A; Amplyx Pharmaceuticals, San Diego, USA) pure substance was stored in aliquots at −80°C, and stock solutions were prepared in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Brøndby, Denmark; 5000 mg/liter). The final drug concentration ranges studied were 0.001 to 0.5 mg/liter. The following comparator compounds were also investigated (source of compound and final concentration range in parentheses): anidulafungin (Pfizer A/S, Ballerup, Denmark; 0.004 to 4 mg/liter), micafungin (Astellas Pharma Inc., Tokyo, Japan; 0.004 to 4 mg/liter), amphotericin B (Sigma-Aldrich; 0.004 to 4 mg/liter), fluconazole (Sigma-Aldrich; 0.03 to 32 mg/liter), and voriconazole (Pfizer A/S, Ballerup, Denmark; 0.004 to 4 mg/liter). Microtiter plates with 2-fold dilutions prepared in double-concentrated medium according to the EUCAST methodology were prepared using serial dilution and pipette tip changes for every fourth well. Cell culture-treated (Nunc MicroWell 96-well microplates; Thermo Fisher Scientific; catalog no. 167008) were used throughout and frozen at −80°C prior to use. The EUCAST quality control (QC) strains *C. albicans* CNM-CL-F8555, *C. parapsilosis* ATCC 22019, and *C. krusei* ATCC 6258 were tested in parallel. MICs for the licensed compounds were classified as wild type and non-wild type, adopting the EUCAST epidemiological cutoff values (ECOFFs) valid 4 February 2020 (https://www.eucast.org).

Data management. MIC ranges, modal MIC (the most common MIC), MIC_{50}, and MIC_{90} values were calculated. Wild-type upper limits (WT-ULs), defined as the upper MIC value where the wild-type distribution ends, were determined for manogepix following principles for setting EUCAST ECOFFs. This includes a visual inspection of histograms of the MICs for single species (the eyeball method) and a statistical method using 97.5% and 99% endpoints and the EUCAST ECOFF finder program (15). However, as the values reported here are not formally accepted EUCAST manogepix ECOFFs, we used the term "WT-UL" to avoid confusion.

Pearson's correlation was used to determine species and antifungal drug-specific MIC correlation. Results were considered significant when the P value was <0.05 and the correlation classified, depending on the r value, as very weak (VW, 0 to 0.19), weak (W, 0.2 to 0.39), moderate (M, 0.4 to 0.59), strong (S, 0.6 to 0.79), and very strong (VS, 0.8 to 1).

ACKNOWLEDGMENTS

We thank research technician Birgit Brandt for excellent technical assistance.

This study was supported by an unrestricted grant from Amplyx Pharmaceuticals. The funder had no influence on the study design or on the analysis of the results.

Outside this work, the authors have the following potential conflicts to declare. K.M.J. has received a meeting grant from MSD and travel grants from Amplyx and F2G. M.C.A. has, over the past 5 years, received research grants/contract work (paid to the Statens Serum Institut) from Amplyx, Basilea, Cidara, F2G, Gilead, Novabiotics, Scynexis, and T2Biosystems and speaker honoraria (personal fees) from Astellas, Gilead, Novartis, MSD, and Seges. She is the current chairman of the EUCAST Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST).

REFERENCES

1. Zhao M, Lepak AJ, VanScoy B, Bader JC, Marchillo K, Vanhecker J, Ambrose PG, Andes DR. 2018. In vivo pharmacokinetics and pharmacodynamics of APX001 against Candida spp. in a neutropenic disseminated candidiasis mouse model. Antimicrob Agents Chemother 62:e02542-17. https://doi.org/10.1128/AAC.02542-17.
2. Hager CL, Larkin EL, Long L, Zohra Abidi F, Shaw KJ, Ghannoum MA. 2018. In vitro and in vivo evaluation of the antifungal activity of APX001A/APX001 against Candida auris. Antimicrob Agents Chemother 62:e02319-17. https://doi.org/10.1128/AAC.02319-17.
3. Miyazaki M, Horii T, Hata K, Watanabe NA, Nakamoto K, Tanaka K, Shirotori S, Murai N, Inoue S, Matsukura M, Abe S, Yoshimatsu K, Asada M. 2011. In vitro activity of E1210, a novel antifungal, against clinically important yeasts and molds. Antimicrob Agents Chemother 55:4652–4658. https://doi.org/10.1128/AAC.00291-11.
4. Pfaller MA, Duncanson F, Messer SA, Moet GJ, Jones RN, Castanheira M. 2011. In vitro activity of a novel broad-spectrum antifungal, E1210, tested against Aspergillus spp. determined by CLSI and EUCAST broth microdilution methods. Antimicrob Agents Chemother 55:5155–5158. https://doi.org/10.1128/AAC.00570-11.
5. Wiederhold NP, Najvar LK, Fothergill AW, McCarthy DI, Bocanegra R, Olivo M, Kirkpatrick WR, Everson MP, Duncanson FP, Patterson TF, Pattersona TF. 2015. The investigational agent E1210 is effective in treatment of experimental invasive candidiasis caused by resistant Candida albicans. Antimicrob Agents Chemother 59:690–692. https://doi.org/10.1128/AAC.03944-14.
6. Pfaller MA, Huband MD, Flamm RK, Bien PA, Castanheira M. 2019. In vitro activity of APX001A (manogepix) and comparator agents against 1,706 fungal isolates collected during an international surveillance program in

2017. Antimicrob Agents Chemother 63:1–11. https://doi.org/10.1128/AAC.00840-19.
7. Arendrup MC, Chowdhary A, Astvad KMT, Jørgensen KM. 2018. APX001A in vitro activity against contemporary blood isolates and Candida auris determined by the EUCAST reference method. Antimicrob Agents Chemother 62:1–9. https://doi.org/10.1128/AAC.01225-18.
8. Morio F, Jensen RH, Le Pape P, Arendrup MC. 2017. Molecular basis of antifungal drug resistance in yeasts. Int J Antimicrob Agents 50:599–606. https://doi.org/10.1016/j.ijantimicag.2017.05.012.
9. Arendrup MC, Patterson TF. 2017. Multidrug-resistant Candida: epidemiology, molecular mechanisms, and treatment. J Infect Dis 216:S445–S451. https://doi.org/10.1093/infdis/jix131.
10. Kapoor M, Moloney M, Soltow QA, Pillar CM, Shaw KJ. 2019. Evaluation of resistance development to the Gwt1 inhibitor manogepix (APX001A) in Candida species. Antimicrob Agents Chemother 64:1–12. https://doi.org/10.1128/AAC.01387-19.
11. Jensen RH, Johansen HK, Søes LM, Lemming LE, Rosenvinge FS, Nielsen L, Olesen B, Kristensen L, Dzajic E, Astvad KMT, Arendrup MC. 2016. Posttreatment antifungal resistance among colonizing Candida isolates in candidemia patients: results from a systematic multicenter study. Antimicrob Agents Chemother 60:1500–1508. https://doi.org/10.1128/AAC.01763-15.
12. Arendrup MC, Meletiadis J, Mouton JW, Guinea J, Cuenca-Estrella M, Lagrou K, Howard SJ, Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). 2016. EUCAST technical note on isavuconazole breakpoints for Aspergillus, itraconazole breakpoints for Candida and updates for the antifungal susceptibility testing method documents. Clin Microbiol Infect 22:571.e1–571.e4. https://doi.org/10.1016/j.cmi.2016.01.017.
13. Pfaller MA, Watanabe N, Castanheira M, Messer SA, Jones RN. 2011. Pre-clinical development of antifungal susceptibility test methods for the testing of the novel antifungal agent E1210 versus Candida: comparison of CLSI and European Committee on Antimicrobial Susceptibility Testing methods. J Antimicrob Chemother 66:2581–2584. https://doi.org/10.1093/jac/dkr342.
14. Chowdhary A, Prakash A, Sharma C, Kordalewska M, Kumar A, Sarma S, Tarai B, Singh A, Upadhyaya G, Upadhyay S, Yadav P, Singh PK, Khillan V, Sachdeva N, Perlin DS, Meis JF. 2018. A multicentre study of antifungal susceptibility patterns among 350 Candida auris isolates (2009–17) in India: role of the ERG11 and FKS1 genes in azole and echinocandin resistance. J Antimicrob Chemother 73:891–899. https://doi.org/10.1093/jac/dkx480.
15. Turnidge J, Kahlmeter G, Kronvall G. 2006. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. Clin Microbiol Infect 12:418–425. https://doi.org/10.1111/j.1469-0691.2006.01377.x.